FOOD GRADE COLLAGEN AS A HAMBURGER EXTENDER

By

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CHAPTER I

INTRODUCTION

Hamburger has developed into an integral part of the American diet. Its consumption has increased steadily over the past few years. An equivalent of half of all the beef from grain-fed steer and heifer carcasses is used for ground beef (Root, 1978), with most of the total being consumed in institutional systems. McDonalds, a fast-food chain, serves nearly 5 million kg of ground beef per week, which contributes significantly to the approximately 23 kg consumption per capita annually in the USA (Pabst, 1979 and Cross & Berry, 1980). Food service as well as hotels, restaurants, and institutional feeding is expanding into world-wide markets and the ground beef patty is becoming commonplace around the world.

Inflationary trends that are taking place with conventional proteins, i.e., meat, milk, and eggs, are causing many researchers to seek other materials to help control the price of products for the consumer. Product development is being pursued toward meat-like, or meat combination products of high quality and acceptance. Vegetable substances, such as soy proteins, have been approved for use as extenders in meat products at a level

not to exceed 3 1/2% of the finished all meat product. Soy products such as soy flour (soy bits, soy grits), soy protein concentrate, and isolated soy protein have been used as meat extenders in the manufacture of a meat patty. In school programs, the Food Nutrition Service of USDA has approved the use of TSP (textured soy protein) at levels as high as 30% (USDA, 1972). The use of soy protein permits a substantial reduction in cost of the finished product. Predictions are that by 1980 approximately 40-50% of all ground beef or processed meat products will contain textured soy protein as a significant ingredient (Whilding, 1974). However, Robinson (1972) reported that 71% of the consumers surveyed were prejudiced against meat analogs even before trying them. Undenatured soluble cheese whey proteins used in ground beef was studied by Jelen (1975), but this product was found to have limited acceptance in human foods due to its insolubility and gritty character.

Hide protein collagen, because of its biophysical properties, is useful as an extender, moisturizer, texturizer or emulsifier in different food systems (Henrickson, 1980). Collagen has been found to be bacteriologically safe for human consumption, bland in flavor and odorless (Whitmore et al., 1970). All of these functional properties make collagen a potential ground beef extender.

The purpose of this study was to determine the effect of adding 0, 10 and 20% food-grade hide protein to hamburger

patties and to evaluate the effect of storage on color, texture, cooking loss, degree of oxidation and sensory evaluation.

CHAPTER II

REVIEW OF LITERATURE

Ground Beef

Ground beef, or chopped beef, can be defined as chopped fresh and/or frozen beef, with or without seasoning and without the addition of fat as such and shall contain no more than 30% fat. It may not contain added water, binder or extenders, but may contain beef cheek not to exceed 25% (de Holl, 1981).

Beef patties, consist of chopped fresh and/or frozen beef with or without the addition of beef fat as such and/or seasonings. Binders or extenders and/or partially defatted beef fatty tissue may be used without added water or with added water only in amounts such that the product's characteristics are essentially that of a meat patty (de Holl, 1981).

In the United States, hamburger or ground beef is a popular meat since it is one of the least expensive beef products available to consumers (Mise, 1972). Today's retail price of ground beef (Regular 75% lean) ranges from 1.22 to 1.28 dollars/pound, compared to the price of a strip loin steak ranging from 6.25 to 6.35 dollars/pound, as referenced in the Meat Price Report, September 1983.

There is great concern for producing a consistent quality product. Since numerous factors can affect the palatability, cooking properties, and ultimate consumer acceptance of ground beef, new technology and research have evolved to assure that ground beef products meet nutritional expectations.

Cross et al. (1976) carried out a study in order to determine how ground beef formulation of varying quality grades and meat cuts would affect cooked ground beef palatability. They reported that ground beef patties from US Utility or Cutter grade carcasses were unacceptably high in connective tissue, whereas ground beef patties from Prime, Choice, and Good grade carcasses were rated as acceptable in all palatability traits. Patties formulated from chucks were rated more desirable in tenderness, flavor, connective tissue amount, and overall acceptability than patties from short plate-chuck combinations. Differences in palatability due to quality grade were larger than those due to cuts.

Since the major sources of lean for ground beef are minor cuts and trimmings from young cattle and major cuts from older animals, it is not economically feasible for the industry to use high priced cuts as the source of lean (Cross et al., 1978). They investigated methods of comminution that would remove a portion of the objectionable connective tissue, and confirm that the new technology called mechanical desinewing would effeciently improve

tenderness in beef patties. Similar results were found by Wells et al. (1980) when they used chilled cow beef.

Cross et al. (1979), found less cooking loss from patties prepared from hot-boned beef, being superior in juiciness and tenderness than those prepared from chilled beef. This fact was confirmed by Cross and Tennet (1981), who also found that the time of boning had a significant effect on total cooking loss, tenderness, and juiciness. As boning time increased from 1 to 24 hours, sensory panel rating for tenderness and juiciness decreased significantly. However, Wells et al. (1980) found that grinding rather than desinewing improved palatability when they used hot-boned beef.

Berry and Stiffler (1981), found that fat loss during cooking was higher in patties made from electrically stimulated than nonstimulated beef, while moisture during cooking was greater for patties from nonstimulated than stimulated beef.

Ground beef research has been concerned with the fat content. Glover (1964) determined that consumers discriminate against ground beef with high fat content because of excessive shrinkage, splattering during cooking, its implications as a cause of obesity, and its greasy taste. In general, previous research indicated that consumers seem to prefer ground beef patties containing 15% fat or more (Law et al., 1971).

Freezing remains the method of choice for long-term

storage during distribution of ground beef even though freezing of meat generally is considered to create tissue damage and some quality loss (Anon & Calvelo, 1980). Freezing treatments for ground beef patties involve rapid freezing techniques in order not to drastically alter palatability, as shown by a consumer acceptance panel (Sebranek et al., 1978). Patties frozen using rapid freezing techniques (N_2 and CO_2) had lower TBA numbers and significantly higher water holding capacity values than patties frozen by a slow air-blast technique (Sebranek et al., 1979).

Collagen

Connective tissue consists of three distinct components: fibrous proteins, ground substances, and cells. The major fibrous proteins are collagen, elastin and reticulin.

About 10 different collagen types have been reported so far (Harwood, 1979). The presence of five types of α chains, namely α1 (I), α 1 (II), α1 (III), α1 (IV), and α 2 chains, are well established in collagen molecules from various sources (Miller, 1973; Epstein, 1974; Johnson et al., 1974; Epstein and Muderloh, 1975; Slutskii and Simkhovich, 1980). These chains constitute various types of collagen which are generally distinct and differ in primary structure.

Type I collagen is composed of two identical α 1 (I)

chains and one $\alpha 2$ chain and is denoted as $\{\alpha 1\ (I)\}_2\ \alpha 2$, found in mature skin, tendon, bone, and cornea. It is the major component of epimysium and perimysium.

Type II collagen, from cartilage, is composed of the identical $\alpha 1$ (I) chains and is designated $\{\alpha 1\ (II)\}_3$. It does not exist in skeletal muscle

Type III collagen, found in human fetal dermis, and the cardiovascular system, is composed of three identical $\alpha 1$ (III) chains and is named $\{\alpha 1\ (III)\}_3$. It was mainly identified in the perimysium and to a lesser extent in the endomysium.

Type IV collagen is found in the basement membrane. This collagen is composed of three identical $\alpha 1$ (IV) chains and is designated $\{\alpha 1\ (IV)\}_3$.

Collagen, a glycoprotein, is the longest of all protein molecules and is composed of tropocollagen monomers which are 300 nm long and 1.5 nm in diameter (Piez, 1967; Woodhead-Galloway et al., 1975; Harwood, 1979). Each tropocollagen monomer comprises three polypeptide α chains, each having a molecular weight of 95,000. The three α chains in a tropocollagen monomer may be identical (collagen types II, III and IV) or different (collagen I). Each chain is coiled into a left-handed helix, with about three amino acids per turn, but the trimers are supercoiled in a right-handed helix (Ramachandran and Ramakrishnan, 1976).

Grassman (1965), stated that the linear polymerization

of tropocollagen monomers produced collagen fibrils which are arranged into parallel bundles (in tendon) or into a three-dimensional irregular network (in skin, cartilage, bone and teeth).

The amino acid composition of collagen is unique in some respects among other proteins. It is extraordinarily rich in glycine, proline and contains large amounts of hydroxyproline, whereas tryptophan is absent. Cysteine is present only in collagen types III and IV, and methionine is the only sulfur containing amino acids in collagen types I and II. Thirty-three percent of the total amino acid residues consist of glycine, about twelve percent of proline, and eleven percent each of alanine and hydroxyproline (Metzler, 1977).

There are two structurally and functionally distinct regions in the collagen chain: a central triple helical regions composed of 1011 amino acids residues and the N- and C- terminal nonhelical regions composed of 9 - 25 residues (Kuhn, 1969). The triple helical regions are composed of chains of tripeptide units of the general formula (Gly - X - Y)n in all types of collagen. However, the distribution of amino acids between X and Y position is uneven (Fietzek and Kuhn, 1976).

The nonhelical regions are devoid of hydroxyproline residue. Sixteen amino acids residues with the same sequence have been found in the N-terminal of the $\alpha 1$ chain of type I collagen from different species. Generally the N-

terminal region is high in hydrophobic amino acids. Investigations on the nonhelical C-terminal and of the α 1(I) chain of collagen from different species have shown the presence of 25 amino acids of which hydrophobic amino acids account for the major proportion.

The peptide linkage formed by the different amino acids (other than proline and hydroxyproline) contain the NH group, which can participate in hydrogen bonding and contributes to the stability of the helix of proteins. However, proline and hydroxyproline serve different purposes in the collagen structure. The N atom of the proline and hydroxyproline residues is linked with α-C to form a rigid five-membered ring structure; hence there is no freedom of rotation about the N-C bond. Hydroxyproline plays a part in the stability of collagen's minor and superhelix by hydrogen bonding, which involve the oxygen of hydroxyproline's hydroxyl group with the backbone of the collagen triple helix via a water dipole (van Hippel, 1967; Ramachandran and Ramakrishnan, 1976). The ability of the chains to attain the triple-helical conformation and its thermal stability depends not only on the content of proline and hydroxyproline residue (Gustavson, 1955; Josse and Harrington, 1964; Sakakibra et al., 1973; Berg and Prockop, 1973; Jimenez et al., 1973; Jimenez and Yankowski, 1975), but also on the distribution of these residues along the chains (Berg and Prockop, 1973).

The distribution of polar and hydrophobic amino acids

residues determine the ordered aggregation of molecules into fibrils (Highberger et al., 1971; Fietzek et al., 1974).

Hydroxylysine that may occur in both the helical and nonhelical N-terminal region, plays an important role in intermolecular cross-linking (Tanzer, 1973; Bailey et al., 1973, 1974). It has been defined to possess four different types of bonds: hydrogen bonds, hydrophobic bonds, ionic bonds and covalent bonds.

Hydrogen bonds are important for stabilizing the secondary structure and packing of collagen molecules (Harrington, 1964); they fix the shape of the protein in a specific conformation. In native collagen, the tropocollagen chains are oriented so that the NH group of the third peptide linkage of an adjacent chain.

Hydrophobic bonds, the side group of other nonpolar amino acids may form inter- and intramolecular hydrophobic bonds in the nonpolar segments (interbands regions) of chains (Schubert and Hamerman, 1968).

Covalent bonds, disulfide linkages, and interchain disulfide bonds have been found in the C-terminal extraglobular peptide region of procollagen chains of all types. It is not present in collagen types I and II due to the absence of cysteine residues in their tropocollagen chains. It has been reported in the helical region of type II collagen (Harwood, 1979) and in the glycoprotein extensions (terminal regions) of type IV collagen (Kefalides, 1973). These types of collagen contain

appreciable amounts of cysteine residues. Cross-linkages involve lysine and hydroxylysine. The intermolecular cross links are formed by a series of aldemine or ketoimine Schiff base and aldol condensation reaction leading to the formation of highly stable compounds (Tanzer, 1976); their amount increases with the age of the animal.

Manufacture of Food Grade Hide Collagen

Hide collagen number four is one of five comminuted products from (18-24 months) cowhide trimmings manufactured by the U.S. Department of Agriculture's Regional Research Center. The process consists of three main operations: precutting, acidifying, and grinding (Komanosky et al., 1974). A flow diagram for the comminution of unhaired, fleshed cattlehide is shown in Figure 1. Limed hides are first sliced in a strip cutter and then cut into small particles in a rotary knife cutter. These precut hide particles are later acidified with the desired organic acid solution (0.3% propionic acid and 0.1% benzoic acid) to the isoelectric point of limed hide (pH = 5.3). Subsequent grinding in the 0.508 cm head of the Urschel Comitol for further sheared in the disc mill, where water is added. This wet product is a whitish fibrous material, bland in flavor and odorless, available in the frozen state in can size number ten.

Collagen must come from inspected slaughter and identity with acceptable carcasses must be established for

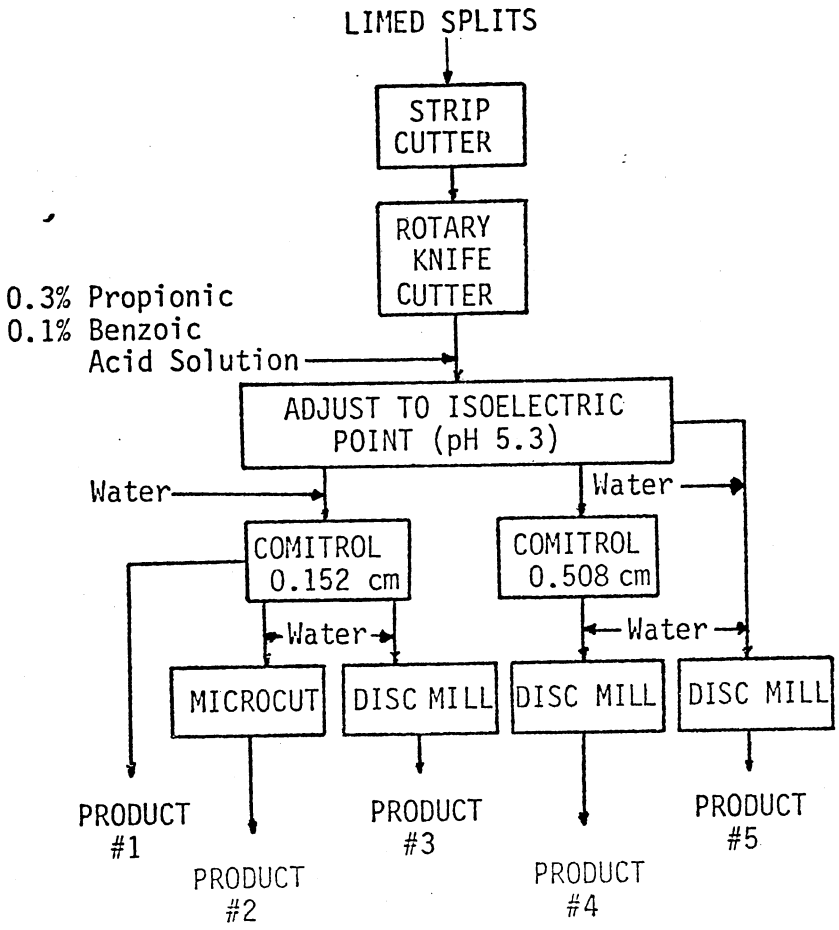

Figure 1. Flow Diagram for the Comminution of Unhaired, Fleshed Cattlehide

all hides intended for food use. Delimed, washed, fibrous insoluble hide collagen when fed to rats was well digested (90%) and served as a source of energy. It was not toxic when fed at 20% collagen for 90 days (Whitmore et al., 1975).

Types of Food Grade Hide Collagen Available

Food grade collagen is available in many forms. USDA Regional Research Center produced in wet form five different comminuted products, varying in particle size and fiber length. All of these were also prepared in the air dried or freeze dried form.

Product Number 1 is composed of densely matted fibers with a relatively large particle or nodule. Product Number 2 consists of smaller particles and is less dense than Product Number 1. Product Number 3 possesses fiber bundles well separated by the shearing action of the disc mill. In product Numbers 4 and 5 the fiber bundles are much shorter and quite airy, having been sheared into individual fibers. In each case the moisture, ash, fat and protein content was variable.

Secol Company (100 North Morehall Road, Malver, Pennsylvania) is commercially producing four products: native dry, hydrolyzed, native wet and a 1% soluble collagen.

Functional Properties of Collagen in Food Systems

The functional properties of protein depend on intrinsic physico-chemical characteristics such as amino acid composition and sequence, molecular weight, conformation, and charge distribution on the molecule. The functional properties of collagen are important for the organoleptic quality of the ultimate product. Proteins are not generally functional in the absence of an aqueous phase; therefore, hydration is the first step in imparting other desirable functional properties such as swelling, gelation, solubility, viscosity, wettability, emulsification, cohesion, adhesion, elasticity and foaming in a food system. Protein holds water in two forms: one is called the bound, structural, or protective form and the other the free or biologically active form. The bound fraction is firmly held as water of hydration by a functional group of the protein in the form of mono and multimolecular layers, having ice-like structure. The free water fraction exists in an ordered form (because of H_2O-H_2O molecule interaction), with motional freedom (Ling and Walton, 1976) or freely mobile (Cooke and Kuntz, 1974).

During hydration the collagen fiber structure is distorted, permitting fiber length and diameter to increase. Two types of collagen hydration are recognized depending on the ionic atmosphere (Gustavson, 1956). The hydration of collagen due to ionic groups and their charges in acid or base is regarded as "osmotic swelling", and the hydration

caused by the interaction of ions of neutral salts or nonionic reagents with nonionic bonds (e.g., hydrogen bond) of collagen is described as "lyotrophic hydration" or "swelling". There are differences in the two types of swelling. Although the osmotic or electrostatic swelling that occurs in dilute acid solutions results in great volume increase, the process is reversible in contrast to the lyotrophic swelling. The osmotic swelling is considered interprotofibrillar, and the integrity of the triple-helical structure of collagen remains intact. On the other hand, the lyotrophic agents may alter the water structure around the collagen fibrils and interrupt the interprotofibrillar structure; hence irreversible changes may occur in the native peptide chains.

Swelling of proteins is an important property in foods such as processed meats, custards, and doughs where protein are required to imbibe and hold water without dissolving. Wettability is another functional property closely associated with hydration and swelling of proteins. It mainly depends on the hydrophobic balance, the molecular surface of the protein, and the surface tension of solvent. These characteristics determine the body and viscosity of some processed meat products (Kinsella, 1979).

Viscoelasticity is the unique physico-chemical property of fibrous collagen that has been utilized in the fabrication of useful products such as edible collagen sausage casings (Braun and Braun, 1956; Reissman and

Nichols, 1960; Cohen, 1964; Talty, 1969; and Kidney, 1970). The manufacture of sausage casing depends on the viscoelastic characteristics of the dispersed collagen. Due to these characteristics, collagen dispersions can serve as a binder and a lubricant.

CHAPTER III

MATERIALS AND METHODS

Food Grade Hide Collagen

Five cans of collagen (Product No. 4), provided by the U.S. Department of Agriculture's Eastern Regional Research Center, were used. This product was prepared January 27, 1979, sealed in number ten size cans and kept at -20°C in the Meat Science Laboratory freezer until used.

Product Number 4 has an average moisture content of 82.9% (Turgot et al., 1978) and was found fully acceptable for food use from the chemical and microbiological standpoints.

Prior to use, each can was held in a 4°C cooler for 48 hours, then opened and placed in a Buchner funnel in a 4°C cooler for 30 minutes to allow the excess moisture to drain. After that, proximate analysis of the collagen samples were made, following Official Methods of the AOAC (1980), for meat and meat products in order to determine the moisture, crude protein (N x 5.56, Henrickson et al., 1983) and crude fat (ether extractable) content.

Preparation of Beef Patties

USDA Good grade beef round and beef fat purchased from

Ralph's Packing Company, Perkins, Oklahoma, were sources for ground beef formulations used throughout this study.

Approximately 10,206.00 g of ground beef were used for each of the five replications. After physically removing the fat, both the fat and lean meat were ground separately once through a 1.27 cm plate using a Globe grinding machine (Model 5028, 1 Hp, 115/230 Volts).

The initial fat content of the lean and the fat portion of the round were measured using the modified Babcock method for meat (Salwin et al., 1955). Three samples were obtained from the lean and fat and an average was computed. The lean and fat were packaged separately into 1134 g in freezer paper and frozen at -15°C until used.

Based on the measured fat percentage, formulations of ground beef were computed to obtain approximately 25% fat in each treatment. The ground lean and fat were each thawed at 4°C for 12 hours. The lean was divided into three batches. For each batch the lean meat was replaced with hide collagen at 0, 10, and 20% while maintaining a 25% fat level in each batch. The amounts of ground beef, ground fat and wet collagen for the formulated beef patties are presented in Table I. All three ingredients were blended using a Hobart mixer for three minutes to insure thorough mixing of the ingredients.

After mixing, each batch was ground through the 0.32 cm plate in order to provide a uniform distribution of the fat, lean and collagen. Both the grinder and mixer were cooled

TABLE I

FORMULATION OF GROUND BEEF PATTIES CONTAINING 0, 10 AND 20% FOOD GRADE HIDE COLLAGEN[1]

Collagen %	Collagen Added g	Ground Beef g	Ground Fat g
0	0	2 551.50	850.50
10	340.20	2 221.30	850.50
20	680.40	1 871.10	850.50

[1]Collagen added as a lean tissue replacement.

in a 4°C room for 12 hours prior to being used to prepare the meat for the patty machine.

Patties weighing approximately 104.1 g ± 0.55 g (diameter of 11 cm and thickness of 0.75 cm) were formed using a Hollymatic 200, patty molding machine (Hollymatic Corporation). Patties were interleaved with a wax coated paper and placed into a tray. Two patties were packaged in a 22.5 cm x 18.5 cm plastic foam tray and overwrapped with a clear polyvinyl chloride oxygen permeable film before being stored at -15°C for up to two weeks.

Chemical Analysis

At 0 day a package was taken at random and one raw patty per treatment was analyzed for fat, moisture and protein content (AOAC, 1980). The patty was reground through a 0.3 cm plate and kept in a whirl-pak bag to prevent moisture loss during preparation. Moisture content was determined as weight loss from a 2 - 3 g sample after drying for 24 hours at 102°C. Extractable lipid was determined as the weight loss of the dried samples after 16 hours of extraction with diethyl ether. The amount of crude protein was determined by the kjeldahl method using a Tecator, Kjeltec auto 1030 analyzer. The percentage of protein was calculated as percentage of nitrogen times 6.25. Triplicate samples from each patty were used to determine the amount of moisture, protein and fat.

Cooking Procedure and Cooking Loss

Using a temperature controlled Toastmaster Deluxe Electric Griddle (Model 875, 1100 watts, 120 volts, A.C.) set at 135°C and preheated for 15 minutes, three weighed patties from each collagen level and on each storage condition were cooked for five minutes on one side and four minutes on the other side to achieve an internal temperature of 65.5°C. Internal temperature was controlled by inserting a meat thermometer into the center of one patty.

After the internal temperature had reached 65.5°C, all patties were removed to allow them to cool to 25°C for 60 minutes. For this purpose they were placed on a cutting board with a wax coated paper. Each patty was weighed to determine the cooking loss. The percentage of cooking loss was calculated by the following formula:

$$\frac{\text{Initial weight - final weight}}{\text{Initial weight}} \times 100$$

One of the three cooked patties was used for the determination of the cooked color, the second for the TBA value and the third for a shear force determination.

Procedure for the Sensory Evaluation

In each session one patty from each treatment was cooked by the procedure previously described before serving to the panelists. Each patty was sectioned into eight

pieces and kept in the Toastmaster set at 65° for five minutes in order to maintain the samples at the serving temperature. They were served as soon as possible to the panelists (Guidelines for Cookery and Sensory Evaluation of Meat, American Meat Science Association, 1977).

At the various storage intervals, a 6 - 9 member panel evaluated patty samples from each treatment for flavor, juiciness, texture and overall acceptability using a 7 point hedonic rating scale as shown in Figure 2.

a. Ground beef flavor intensity: 7 = intense beef flavor, 1 = extremely off flavor
b. Juiciness: 7 = extremely juicy, 1 = very dry
c. Texture: 7 = extremely cohesive, 1 = very crumbly
d. Overall acceptability: 7 = like extremely, 1 = dislike extremely

Panel members, Food Science graduate students from the Animal Science Department at Oklahoma State University, were given instruction on the interpretation of the rating scale prior to the actual testing. They were instructed to chew the sample and then spit out the residue. Panelists were provided with water for oral rinsing between samples and white bread for removing flavor carryover.

The panel was set as suggested in the Laboratory Method for Sensory Evaluation of Food by Research Branch, Canada Department of Agriculture, publication No. 1637, 1977. Samples to be evaluated in each session were selected using a table of random numbers. Panelists rated three coded

SENSORY EVALUATION SCORE SHEET

Name_____

Evaluate the cooked beef patty for flavor, juiciness, texture and overall acceptance, and give your numerical rating.

Sample Code_____ Date_____

Flavor_____

1 - Extremely off flavor
2 - Moderate off flavor
3 - Slight off flavor
4 - Bland, no flavor
5 - Slight beef flavor
6 - Moderate beef flavor
7 - Intense beef flavor

Juiciness_____

1 - Very dry
2 - Moderately dry
3 - Slightly dry
4 - Neither dry nor juicy
5 - Slightly juicy
6 - Moderately juicy
7 - Extremely juicy

Texture_____

1 - Very crumbly
2 - Moderately crumbly
3 - Slightly crumbly
4 - Neither crumbly nor cohesive
5 - Slightly cohesive
6 - Moderately cohesive
7 - Extremely cohesive

Overall Acceptance_____

1 - Dislike extremely
2 - Dislike moderately
3 - Dislike slightly
4 - Neither dislike nor like
5 - Like slightly
6 - Like moderately
7 - Like extremely

Figure 2. Sensory Evaluation Score Sheet

samples at each of 45 sessions.

Objective Analysis for Color

Color of raw and cooked patties was evaluated using a Hunter Lab Tristimulus Colorimeter (Model D25 L-9) following the procedure for meat products described by Hunter (1976). Color was evaluated using L (lightness-darkness), a (redness-greenness), and b (yellowness-blueness) values. The exposed surface was allowed to oxygenate for approximately 30 minutes at room temperature. After oxygenation the surface was blotted dry and presented to the specimen port of the optical sensor (Snyder, 1964). Triplicate L, a, and b readings were taken at each of the three different areas of each patty.

Instron Shear Analysis

An Instron Universal Testing Instrument, Model 1122 with a LEE Kramer Shear Cell was used as an objective measure for texture (Kastner et al., 1973; Falk, 1974; and Schalk, 1980). One cooked patty for each treatment was cooled to 25°C for 60 minutes, then three cores were taken from each patty. The core samples were removed by hand using a coring device with a diameter of 2.54 cm. The rate of crosshead descent and chart speed were calibrated at 100 mm/min. The full scale load was set at 5. Data were recorded for maximum shear force and weight of each core to the nearest 0.01 gram.

TBA Value

Thiobarbituric acid values were determined for raw and cooked patties by the extraction procedure described by Kuntapanit et al. (1978). Reagents were freshly prepared and kept refrigerated (5°C) prior to every TBA determination. At the time of analysis, samples were cut into 0.25 to 0.5 cm cubes, then pulverized in liquid nitrogen to insure muscle sample homogeneity. A ten gram meat sample plus 15 ml of cold (5°C) extracting solution (10% perchloric acid) and 20 ml of deionized distilled water, were blended at high speed (16,000 rpm) for 10 seconds in an OMNI Mixer (Model 1750, 115 Volts, 5 amps). The slurry was filtered through Whatman number two filter paper. Five ml of the filtrate was transferred to a test tube to which 5 ml of 0.02 M of two thiobarbituric acid (TBA) Reagent was added. The test tube was covered with parafin film and hand mixed, then stored in the dark at 25°C for 15 hours. The absorbance was determined using a Gilford Spectrophotometer at 529.5 nm. With each group of meat samples TEP (1,1,3,3,-tetraethoxypropano) standards were run in order to provide a standard curve.

Preparation of the Standard Curves

Standard curves were prepared with each TBA determination in order to minimize errors (i.e., electricity fluctuations, minor technique, etc.). They were prepared from appropriate dilutions of 1×10^{-7} moles of TEP/5 ml

stock solution to give the required concentration of 1 x 10^{-9} to 1 x 10^{-8} moles of TEP/5 ml. Five ml of TEP solution and 5 ml of TBA were placed into a test tube and stored in the dark at 25°C for 15 hours. The absorbance units obtained from the standards were plotted against TEP concentrations. Regression equations were used to calculate TBA value of samples. TBA values were expressed as mg of malonaldehyde per 5 ml filtrate or per 1 kg of sample. Analyses were performed in triplicate.

Statistical Analysis

A randomized block design was used for analyses of fat, protein, and moisture content of raw patties on the three levels of collagen replacements. Three observations from each treatment were taken. Individual cans of product number four utilized in this study were analyzed for fat, protein and moisture content using a complete randomized design.

For the analyses of the sensory variables for the taste panel, a split-plot model in a randomized block design was used. Panelists were the main unit treatment and collagen level and storage week were subunit treatment factors.

For cooked and uncooked color, cooked and uncooked TBA value, and shear force data, a randomized block design with factorial arrangement for treatments was used. Collagen level and storage weeks were the factors involved. Three subsamplings per treatment were used.

The statistical analyses for cooking loss data were evaluated using a randomized block with factorial arrangement. In this case one observation of three patties per treatment was taken.

Calculations for the analysis of variance for the complete randomized design, randomized block with factorial arrangement for treatment, and randomized block design with split-plot model, were accomplished by use of the Statistical Analysis System (Barr et al., 1976); and Steel and Torrie (1980). Mean separation on results from the taste panel was accomplished using the methods of Duncan (1955).

CHAPTER IV

RESULTS AND DISCUSSION

Chemical Analysis

The effect of collagen level on fat (ether extractable), moisture and crude protein content of raw ground beef patties is shown in Tables VIII, IX and X (see Appendix), while Table II shows the mean values of each chemical parameter.

Collagen level (Table VIII, Appendix) did not have a significant effect on the fat content of the patties (P = 0.09), having a mean fat level of 25,12, 25.38 and 25.39% for the 0, 10 and 20% collagen replacement for lean meat, respectively (Table II).

The crude protein content (Table IX, Appendix), was not significantly different at the three collagen levels utilized in this study (P = 0.09). The mean crude protein content of the 0, 10 and 20% ground beef made by replacing lean with collagen was 16.04, 15.92 and 15.88% (Table II).

No significant variation was found in the moisture content (Table X, Appendix) of the ground beef patties (P = 0.47) at the three levels of collagen replacement. The mean values for moisture were 56.37, 56.18, and 56.54% for 0, 10 and 20% collagen replacement (Table II).

TABLE II

CHEMICAL ANALYSIS OF GROUND BEEF PATTIES[1] AS INFLUENCED BY COLLAGEN LEVELS

Collagen Level	Fat %	Crude Protein %	Moisture %
0	25.12	16.04	56.37
10	25.38	15.92	56.18
20	25.39	15.88	56.54

[1]Means from 15 observations

TABLE III

CHEMICAL ANALYSIS OF COLLAGEN[1]

Can No.	Fat %	Crude Protein %	Moisture %
1	0.30	20.54	77.33
2	0.37	19.80	79.10
3	0.36	19.55	79.02
4	0.33	19.92	69.02
5	0.39	19.65	79.55
$\bar{X}$	0.35	19.89	76.80

[1]Means from 15 observations

Since five cans of collagen, product No. 4, were utilized for the purpose of this study, the chemical analysis of each can was performed to determine whether variability was introduced into the ground beef due to the added collagen. Tables XI, XII and XIII (see Appendix) show the analysis variance for the moisture, protein and fat of the collagen in the five cans. The mean value was 0.35% for fat, 19.89% for crude protein and 76.80% for moisture (Table III).

These results indicate that the chemical composition of the final product was essentially the same and therefore valid comparisons can be made on the effects of collagen when added to ground beef patties.

Taste Panel

Flavor, juiciness and overall acceptability were measured during 46 tasting sessions with 6 - 9 semitrained panelists.

Results of the analysis of variance for sensory attributes measured are recorded in Table XIV (see Appendix) for flavor, Table XV (Appendix) for juiciness, Table XVI (Appendix) for texture and Table XVII (Appendix) for overall acceptability. A significant difference ($P<0.05$) was found for flavor, juiciness, texture and overall acceptability due to collagen level. However, no significant difference ($P>0.05$) was found for these attributes due to storage.

Collagen level and storage period interaction

significantly ($P<0.05$) affected the palatability attributes: juiciness, texture and overall acceptability, whereas no significant interaction was found for flavor.

A significant variation ($P<0.05$) was found between panelists for the variable juiciness, which showed that panelists were not in agreement when they scored the samples for this attribute.

Mean values for sensory traits as affected by collagen levels are given in Table IV. Mean flavor scores were significantly higher for patties at 0 level of collagen 5.39, compared to 4.67 at 10% collagen and 3.18 and 20% collagen level. These results indicate that the flavor of the ground beef decreased as the collagen content increased.

Mean sensory panel juiciness scores ($P<0.01$) were higher in patties made with 10% level of collagen having a mean value of 4.89 followed by 20% collagen with 4.53 and by 0% collagen with a mean score of 4.38. This increase in juiciness due to the addition of collagen may be attributed to higher water holding capacity of collagen.

Panel scores for texture were higher ($P<0.01$) in patties made with 0 level of collagen having a mean value of 4.96, a mean value of 3.78 for 10% collagen and a mean score of 2.84 for patties with 0% collagen. As the collagen level increased the texture became less cohesive, leading to a tendency for the patty to crumble.

Scores for overall acceptability were significantly higher ($P<0.01$) for 0% level of collagen having a mean score

TABLE IV

SENSORY VARIABLES FROM TASTE PANEL
AS AFFECTED BY COLLAGEN LEVEL

Collagen Level %	Sensory Panel Rating [1] Flavor[2]	Juiciness[2]	Texture[2]	Overall Acceptability[2]
0	5.39a	4.38a	4.96a	5.00a
10	4.67b	4.89b	3.78b	4.78b
20	3.18c	4.53a	2.84e	2.92e

[1]7 point hedonic scale, with 7 being the highest score
[2]Mean of 45 sessions with six panel members
Means in a column which are not followed by the same letter are significantly different ($P<0.05$)

TABLE V

SENSORY VARIABLES FROM TASTE PANEL
AS AFFECTED BY STORAGE PERIOD

Storage Week	Sensory Panel Rating [1] Flavor[2]	Juiciness[2]	Texture[2]	Overall Acceptability[2]
0	4.46a	4.65a	3.82a	4.24a
1	4.35a	4.68a	3.86a	4.20a
2	4.43a	4.48a	3.90a	4.27a

[1]7 point hedonic scale with 7 being the highest score
[2]Mean of 45 sessions with six panel members
Means in a column which are not followed by the same letter are significantly different ($P<0.05$)

value of 5, a mean value of 4.78 for 10% collagen and 2.92 for 20% collagen level, which indicated that as the collagen level increased the acceptability of the patties decreased. The lowest mean score was defined as dislike slightly. At no time were the samples scored as extremely undesirable.

Mean values for sensory variables from taste panel as affected by storage period are presented in Table V.

These data provide evidence that beef patties prepared with collagen were superior in texture and juiciness when compared to a control prepared with no added collagen, but the flavor and overall acceptability decreased as the level of collagen increased.

Cooking Loss

The effect of collagen level and storage period on the cooking loss of the ground beef patties is shown in Table VIII (see Appendix). Neither of these factors significantly affected the cooking loss. The interaction of collagen level and storage period did not produce significant differences ($P = 0.39$) in cooking loss.

The mean values for cooking loss as influenced by collagen level (Table VI) was 30.56% at the 0% collagen level, 30.45% at 10% collagen and 30.68% at 20% collagen level. The mean values for cooking loss as affected by storage (Table VII) was 30.61% at 0 weeks of storage, 30.50% at 1 week and 30.58% at two weeks of storage. These mean values represent the average across all collagen levels.

TABLE VI

COOKING LOSS, TBA VALUE, COLOR AND TEXTURE AS AFFECTED BY COLLAGEN LEVEL[1]

Collagen Level %	Cook[2] Loss %	TBA Value[23]		Color[2]						Texture[2] (kg/g)
				Cooked			Uncooked			
		Cooked	Uncooked	L	a	b	L	a	b	
0	30.56	2.50	1.99	40.13	5.38	11.36	48.85	13.48	11.61	3.67
10	30.45	2.11	1.53	40.20	5.48	11.30	51.48	11.66	13.87	3.48
20	30.68	1.67	1.13	41.33	6.00	11.24	53.53	11.15	12.26	3.26

[1]Means for each collagen level represent the average across all storage periods

[2]Means from 135 observations

[3]Concentration of malonaldehyde (mg/kg of sample)

TABLE VII

COOKING LOSS, TBA VALUE, COLOR AND TEXTURE AS AFFECTED BY STORAGE[1]

Storage Time (Week)	Cook[2] Loss %	TBA Value[23]		Color[2]						Texture[2] (kg/g)
				Cooked			Uncooked			
		Cooked	Uncooked	L	a	b	L	a	b	
0	30.61	2.03	1.57	39.51	6.07	11.31	50.30	12.16	12.70	2.97
1	30.50	2.46	1.59	41.57	5.75	11.36	51.69	13.15	11.60	3.51
2	30.58	1.79	1.50	40.55	5.05	11.22	51.99	10.84	13.48	3.93

[1]Means for each storage period represent the average across all collagen levels

[2]Means from 135 observations

[3]Concentration of malonaldehyde (mg/kg of sample)

These results are in agreement with Gielissen (1981), who found that weight loss of fine emulsion bologna upon cooking was not significantly affected by collagen replacement. It can be concluded that the collagen added to ground beef did bind moisture during cooking.

Objective Color

The analysis of variation for Hunter L values, which expressed lightness-darkness, on the uncooked patties is presented in Table IX (see Appendix). This showed a significant ($P<0.01$) variation due to storage time. A significant ($P<0.01$) difference was found due to the collagen replacement level with an interaction ($P = 0.02$) due to the treatment factors. Corresponding mean Hunter L values were: 48.85 for 0%, 51.48 for 10%, and 53.53 for 20% collagen level (Table VI), showing an increase in the mean L value as the collagen level increased. Since the Hunter L value has a standard of 0 for black and 100 for white, an increase in this value means that as the lean meat is removed and replaced with collagen the patty lightens in color. Regarding the storage time, an increase ($P<0.01$) in the mean Hunter L value was observed as the storage time increased; thus, 50.20 for 0 week, 51.69 for one week, and 51.99 for two weeks of storage (Table VII). The increase of Hunter L values during storage may be expected since meat will discolor during storage (Snyder, 1964) due to a decrease in partial pressure of oxygen, metmyoglobin

formation and/or bacterial growth which can deprive meat of oxygen.

For the Hunter a (redness-greenness) value of the uncooked patties, the analysis of variation is presented on Table XX (see Appendix), which showed no significant differences (P = 0.35) due to the collagen level of replacement or for the storage period (P = 0.39) and interaction due to the treatments (P = 0.58). Hunter a mean values decreased as the collagen level increased: 13.48 for 0%, 11.66 for 10%, and 11.55 for 20% of the collagen level. The Hunter a mean values for the storage period were: 12.26 for 0 week, 13.15 for one week, and 10.88 for two weeks of storage. However, these differences were not statistically significant.

For the Hunter b (blueness-yellowness) value of the uncooked patties (Table XXI, Appendix), no significant (P = 0.45) variation due to the collagen level of replacement were observed, followed by no significant (P = 0.57) variation for storage time, and for the interaction between collagen level and storage (P = 0.50). Hunter b mean values attributed to collagen level were: 11.61 for 0% collagen, 13.87 for 10% collagen, and 12.26 for 20% collagen. Hunter b mean values due to storage time were: 12.70 for 0 week, 11.60 for one week, and 13.48 for two weeks of storage. However, these variations were not significantly different. Since blueness-yellowness standard values correspond to -50 to +70, an increase in this value indicated that the product

became yellowish when collagen was added. The tendency to increase during storage was also supported by discoloration of fresh meat due to time of storage (Snyder, 1964).

The analysis of variance for Hunter L values for the cooked ground beef patties is contained in Table XXII (see Appendix) Hunter L values were not significantly ($P = 0.07$) affected by the collagen level. Storage time did not significantly ($P = 0.35$) affect the L value. No significant ($P = 0.10$) interaction was found due to the treatments implicated. Mean values for L as affected by collagen were: 40.13 for 0% collagen, 40.20 for 10% collagen and 41.33 for 20% collagen level. Mean values relating to the storage for cooked patties were: 39.51 for 0 week, 41.57 for one week and 40.55 for two weeks of storage, showing the tendency to increase the L color value due to collagen and storage. Gielissen (1981) found that replacing meat with collagen in a fine bologna emulsion up to 12.8% gave no significant variation in the L value. However, Schalk (1981) found significance when collagen was added up to 22.5% in a coarse bologna product. These meat products both contained sodium erythorbate and sodium nitrite which when cooked in the product gave the pinkish color of cured meat, different from that of ground beef without these chemicals.

Table XXIII (see Appendix) contains the analysis of variation for the Hunter a color value for the cooked patties. The Hunter a values were significantly ($P<0.01$) affected by the collagen level. Storage time significantly

($P<0.01$) affected a color values, but no interaction ($P = 0.23$) was found due to these parameters. The mean value for each collagen level were: 5.38 (0% collagen), 5.48 (10% collagen) and 6.00 (20% collagen). The average Hunter a color mean values for storage time across all collagen levels were 6.07 (0 week), 5.75 (one week) and 5.05 (two weeks).

Table XXIV (see Appendix) contains the analysis of variation for b values for cooked ground beef patties. No statistical significance ($P = 0.59$) was observed when b values were evaluated by collagen level. Increasing storage time was not found to significantly ($P = 0.46$) affect the Hunter b value. No significant variation ($P = 0.14$) in b value was found due to the interaction of collagen level and storage period. The average b values for collagen level across all storage periods was 11.36 (0% collagen level), 11.30 (10% collagen level) and 11.24 (20% collagen level). The mean values due to storage period were 11.31 (0 week), 11.36 (one week) and 11.22 (two weeks).

Since color of cooked meat is a result of measurement of these three values (L, a and b) (Hunter, 1976), having a significant variation among one of these values results in a variation of color in the final product. The variation in color values on cooked patties due to collagen level was expected since the color of cooked meat depends upon pigment level, degree of myoglobin denaturation, iron oxidation, the decomposition and polymerization of carbohydrates, fats and

protein (Weir, 1960). Even though the patties at the three collagen levels had characteristics similar in terms of protein, moisture and fat (see chemical analysis), collagen lacks the heme pigment myoglobin.

Instron Shear Force

Tenderness, the main attribute associated with meat texture, was measured by the Kramer shear force and expressed as kg/g of ground beef patty. The analysis of variance presented in Table XXV (see Appendix) showed a significant difference ($P<0.05$) between the blocks or replications of the experiment. A significant tenderness variation ($P<0.05$) was found due to collagen replacement.

The mean value of the shear force as affected by the collagen level is shown in Table VI. As the level of collagen increased, 0, 10 and 20%, the shear force tended to decrease: 3.76, 3.48 and 3.27 kg/g, respectively. Figure 3 shows the effect of collagen level on shear force during storage.

A significant variation in texture ($P = 0.01$) was observed due to the storage period treatment. The mean values for the shear force, as affected by the storage time, showed a tendency to increase (Table VII). Corresponding to 0, one and two weeks of storage time, the mean shear force was 2.97, 3.51 and 3.93 kg/g, respectively. The interaction of collagen level and storage period did not produce significant differences ($P = 0.12$) in shear force.

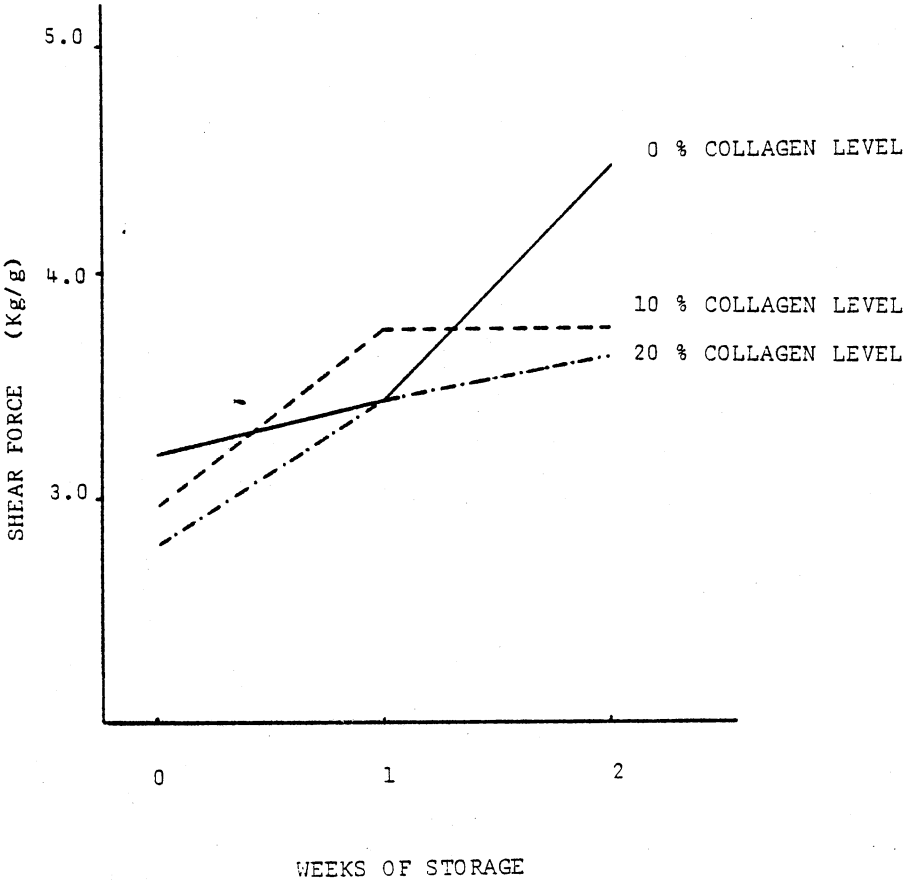

Figure 3. Effect of Collagen Level on Shear Force During Storage

Instron shear force results indicated that upon collagen replacement the product tends to become less cohesive, decreasing the texture of the product. Similar results were reported by Schalk (1981) in a coarse bologna product, due to gelatinization of the collagen. The significant increase of mean shear force due to the length of the storage period may reflect hardening of the collagen and muscle fibers, increasing the cohesiveness or internal bonding strength of the meat.

TBA Value

The TBA analysis was performed on cooked and uncooked patties, and the results were expressed as the concentration of malonaldehyde (mg/kg of sample) for the index of rancidity (Tables XXVI and XXVII, Appendix).

Collagen level significantly ($P = 0.05$) affected the TBA value of the uncooked patties. The mean TBA values for 0, 10 and 20% replacement of lean meat were: 1.99, 1.53 and 1.13 mg/kg, respectively, which indicated that as the collagen replacement of lean meat was increased, a lower concentration of malonaldehyde was obtained. Figure 4 shows the effect of collagen level on TBA value for uncooked patties during storage.

No significant variation ($P = 0.87$) was found due to the period of storage used. No significant interaction between collagen level and storage period was computed.

The mean TBA values for uncooked patties, as affected

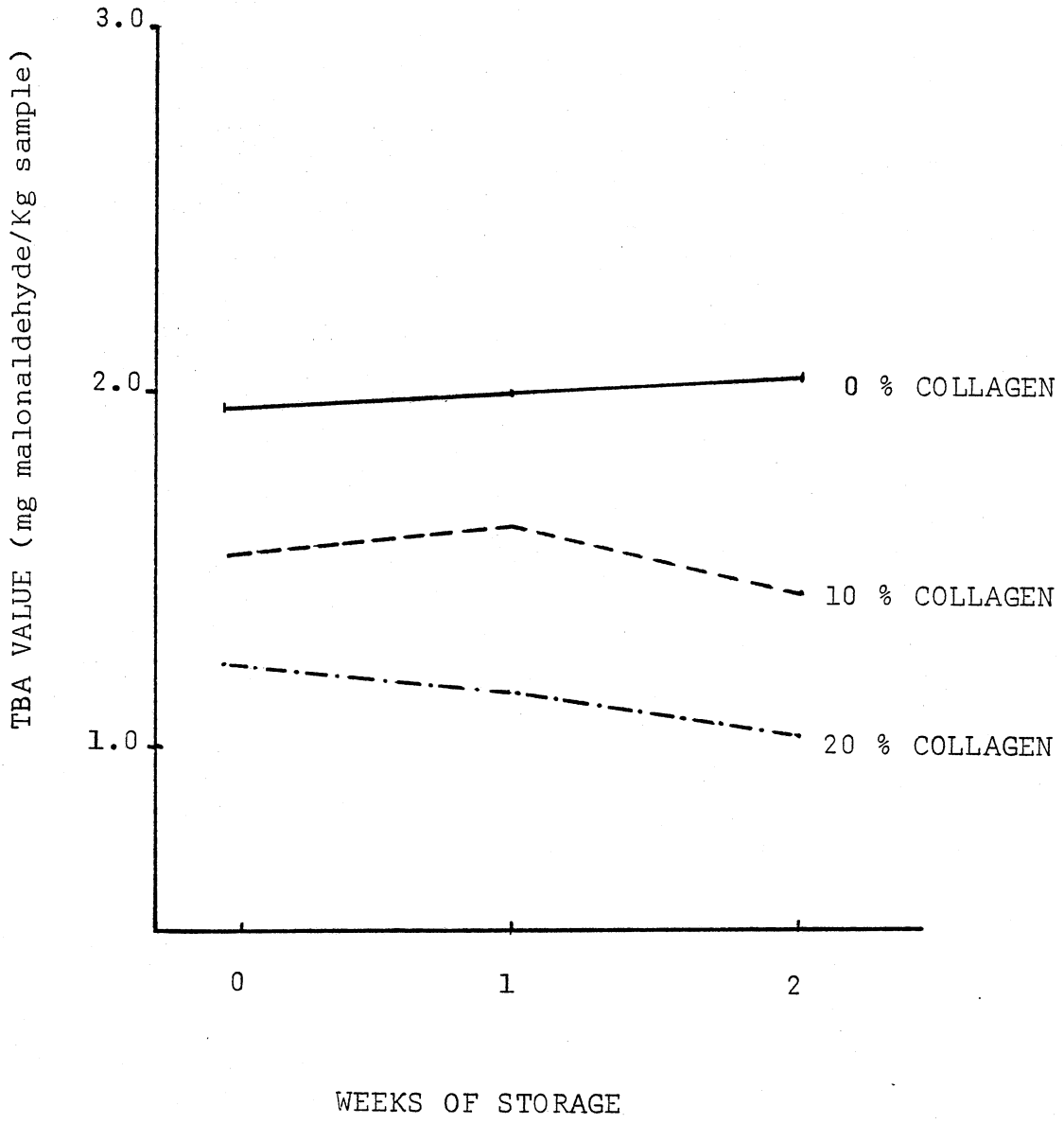

Figure 4. Effect of Collagen Level on TBA Value for Uncooked Patties During Storage

by storage for all collagen levels, were 1.57, 1.59 and 1.50, which showed that the concentration of malonaldehyde increased after the first week with a decrease after the second week of storage. However, these differences were small and not significant.

The development of rancidity, as measured by the TBA test, was less for patties containing 20% of collagen. This can be explained since lean meat has been removed. Tappel (1952, 1953 and 1955) demonstrated that hematin compounds such as myoglobin catalyzed oxidation of unsaturated lipids and this catalytic activity is completely dependent on the presence of iron. The decreasing of lean tissue is responsible for the decrease in TBA value, decreasing the development of rancidity in the product. The increase in TBA value due to storage was due to the oxidation of unsaturated fatty acids, which were accelerated by the presence of oxygen as reported by Kuntapanit (1978), who recommended vacuum packaging. The increase in TBA value at the first week and a decrease at the second week, was supported by Caldironi and Bazan (1982), who reported that bovine muscle showed a decline in TBA numbers after the initial rise during storage at low temperatures. He explained this as being due to the formation of less stable and/or volatile compounds which react with TBA. Dugan (1961), Moledina et al. (1977), and Gokalp et al. (1978) reported that some metabolites may be susceptible to oxidation yielding products unreactive with TBA reagent.

For the cooked patties, the analysis of variation for TBA values are shown in Table XXVII (see Appendix). Collagen level significantly ($P<0.01$) affected the TBA value. Mean values (Table VI) were 2.50, 2.11 and 1.67 mg of malonaldehyde/kg of sample for the 0, 10 and 20% collagen replacement levels. In this case a significant ($P<0.01$) variation was found for TBA value in the cooked patties due to storage week. The mean TBA values in ground beef patties as affected by storage were 2.03, 2.46 and 1.79 mg/kg of sample (Table VII). No significant interaction was found due to collagen level and storage for the TBA value for cooked ground beef patties. The higher TBA values in cooked patties when compared with raw patties indicated that temperature accelerated the oxidation.

CHAPTER V

SUMMARY AND CONCLUSIONS

Ground beef patties containing 0, 10 and 20% Food Grade collagen were stored at -15°C for up to two weeks to evaluate the effect of collagen level and storage period on quality characteristics. Subjective evaluation was made by a semitrained panel to evaluate various quality attributes: flavor, juiciness, texture and overall acceptability. Objective measurements were made for: color, texture, TBA and cooking loss. When collagen was added to ground beef, the fat, moisture and protein of the final product was not significantly affected.

Subjective data obtained from the panel suggested that beef patties prepared with collagen were superior in texture and juiciness to beef patties with no added collagen, but overall acceptability decreased as the level of collagen increased. The semitrained panel could detect significant difference in flavor, juiciness, texture and overall acceptability due to the collagen level; however, no significant differences were found for these attributes due to storage time. Collagen added to ground beef at 10 and 20% levels did bind moisture during cooking.

An increase in collagen caused a lighter colored patty,

due to the significant decrease in the L value.

Instron shear force indicated that the product became less cohesive due to collagen replacement; however, a significant increase in cohesiveness due to storage suggested that hardening of collagen and muscle fibers during storage may occur.

Replacement of lean tissue by collagen significantly decreased the development of rancidity. Food Grade collagen would reduce cost, because it is less expensive than beef, and it would supplement the consumption of red meat during periods of shortage.

LITERATURE CITED

American Meat Science Association. 1978. Guidelines for cookery and sensory evaluation of meat. Published by AMSA and the National Live Stock and Meat Board, Chicago, IL.

A.O.A.C. 1980. "Methods of Analysis". Association of Official Analytical Chemists, Washington, D.C., 13th edition.

Anon, M. C. and A. Calvelo. 1980. Freezing rate effects on the drip loss of frozen beef. Meat Science 4:1.

Berg, R. A., and D. J. Prockop. 1973. The thermal transition of a non hydroxylated form of collagen. Evidence for a role for hydroxyproline in stabilizing the triple helix of collagen. Biochemical Biophysic Research Commun. 52:115.

Berr, J. A. and Goodnight, J. H. 1972. Statistical Analysis System (SAS). Department of Statistics, North Carolina State University, Raleigh, N. C.

Berry, B. W., and D. M. Stiffler. 1981. Effects of electrical stimulation, boning temperature, formulation and rate of freezing on sensory cooking, chemical and physical properties of ground beef patties. J. Food Science 46:1103.

Braun, E., and B. Braun. 1956. Production of collagen strands. U.S. Patent 2,747,228.

Canada Department of Agriculture. 1977. Laboratory methods for sensory evaluation of food. Publication 1637.

Caldironi, H., and N. Bazan. 1982. Effects of antioxidants on malonaldehyde. Production and fatty acid composition in pieces of bovine muscle and adipose tissue stored fresh and frozen. J. Food Science 47:1329.

Cohen, J. 1964. Benzoic acid treatment of collagen fibers and chrome tannage thereof. U.S. Patent 3,126,433.

Cooke, R., and Kuntz, I. D. 1974. The properties of water in biological systems. Annual Review Biophysics Bioengineering 3, 95.

Cross, H. R., B. W. Berry, J. E. Nichols, R. S. Elder and J. A. Quick. 1978. Effect of desinewing versus grinding on textural properties of beef. J. Food Science 43:1507.

Cross, H. R., B. W. Berry, and D. Muse. 1979. Sensory and cooking properties of ground beef prepared from hot and chilled beef carcasses. J. Food Science 44:1432.

Cross, H. R., and B. W. Berry. 1980. Factors affecting palatability and cooking properties of ground beef patties--frozen lean, patty size, and surface treatment. J. Food Science 45:1463.

Cross, H. R., E. C. Green, M. Stanfield, and W. J. Franks. 1976. Effect of quality grade and cut formulation on the palatability of ground beef patties. J. Food Science 41:9.

Cross, H. R., D. Muse, and E. C. Green. 1979. Effects of precooking beef patties on palatability cooking properties and storage stability. J. Food Science 44:870.

Cross, H. R., and I. Tennent. 1981. Effect of electrical stimulation and postmortem boning time on sensory and cookery properties of ground beef. J. Food Science 46:292.

de Holl, J. C. 1981. Encylopedia of Labeling Meat and Poultry Products. Meat Plant Magazine, 5th edition.

Dugan, L. R. 1961. Development and inhibition of oxidative rancidity in foods. Food Technology 15:10.

Duncan, D. B. 1955. New multiple range and multiple F tests. Biometrics 11:1.

Epstein, E. H., and N. H. Munderloh. 1975. Isolation and characterization of CNBr peptides of human {α I(III) 3 collagen and tissue distribution of{αI(I)} 2 α 2 and { α I(III)}3 collagens. J. Biological Chemistry 250:9304.

Falk, S. N. 1974. Feasibility of "hot" processing the bovine carcass. M.S. thesis, Oklahoma State University, Stillwater, Oklahoma.

Fietzek, P. P., and K. Kuhn. 1974. The covalent structure of collagen: Amino acid sequence of the N-terminal region of 2-CB3 from calf skin collagen. Hoppe-Seyler's Z. Physiology Chemistry 335:647.

Gielissen, R. 1981. The use of bovine hide collagen in coarse bologna. M.S. thesis, Oklahoma State University, Stillwater, Oklahoma.

Glover, R. S. 1964. Consumer preferences for ground beef and implications for cattle producers and beef distributors. Ph.D. dissertation, Texas A & M University, College Station, Texas.

Gokalp, H. V., H. W. Ockerman, R. F. Plimpton, N. A. Parret, and U. R. Cahill. 1978. Effect of different packaging methods on objective quality characteristics of frozen and stored cow beef. J. Food Science 43:297.

Grassmann, W. 1965. Structure and chemistry of collagen. In "Structure and function of connective and skeletal tissue" (S. Fitton-Jackson, ed.), p. 1. Butterworth, London.

Gustavson, K. H. 1955. The function of hydroxyproline in collagens. Nature (London) 175:70.

Gustavson, K. H. 1956. "The chemistry and reactivity of collagen," pp. 102, 155, 171, 202. Academic Press, New York.

Harrington, W. F. 1964. On the arrangement of the hydrogen bonds in the structure of collagen. J. Molecular Biology 9:613.

Harwood, R. 1979. Collagen polymorphism and messenger RNA. Int. Review Connective Tissue Research 8:159.

Henrickson, R. L., and H. Turgut. 1983. Emulsion capacity and stability of beef with various collagen additives. Unprinted.

Henrickson, R. L. 1980. Hide collagen as a food. Symposium. New uses untanned hide collagen. American Leather Chemistry Association Convention. J. American Leather Chemistry Association 75(11):464.

Highberger, J. H., A. H. Kang, and J. Gross. 1971. Comparative studies on the amino acid sequence of the 2-CB2 peptides from chick and rat skin collagen. Biochemistry 10:610.

Hunter, R. S. 1976. Objective methods for appearance evaluation. In: Objective Methods for Food Evaluation. National Academy of Science. Washington, D.C.

Jelen, P. 1975. Use of coagulated lactalbumin from cheese whey in ground beef. J. Food Science 40:1072.

Jimenez, S. A., and R. Yankowski. 1975. Hydroxyproline is not required for secretion of triple-helical collagen. Federal Proceeding, Federal American Society Experimental Biology 34, Abstract No. 1944.

Jimenez, S. A., M. Harsch, and J. Rosenbloom. 1973. Hydroxyproline stabilizes the triple-helix of chick collagen. Biochem. Biophysical Research Community 52:106.

Johnson, A. R., E. Chung, E. J. Miller. 1974. Cited in Miller. 1974.

Josse, J., and W. F. Harrington. 1964. Role of pyrrolidine residues in the structure and stabilization of collagen. J. Molecular Biology 9:269.

Kastner, C. L., R. L. Henrickson, and R. D. Morrison. 1973. Characteristics of hot boned bovine muscles. J. Animal Science 36:484.

Kefalides, N. A. 1973. Structure and biosynthesis of basement membranes. Int. Review Connective Tissue Research 6:63.

Kidney, A. J. 1970. Method of preparing a collagen sausage casing. U.S. Patent 3,505,084.

Kinsella, J. E. 1979. Functional properties of soy proteins. J. American Oil Chemistry Society 56(3):242.

Komanosky, M., H. I. Sinnamon, W. K. Heiland, and N. C. Aceto. 1974. Production of comminuted collagen for novel applications. J. American Leather Chemistry Association 69:410.

Kuhn, K. 1969. The structure of collagen. Essays Biochem. 5:59.

Kuntapanit, C. 1978. Beef muscle and adipose lipid deterioration as affected by nutritional regime, vacuum aging, display and carcass conditioning. Ph.D. dissertation, Kansas State University, Manhattan, Kansas.

Law, H. M., S. P. Young, and A. M. Mullins. 1971. Ground beef quality at the retail level. A study in Baton Rouge, La. J. American Diet Association 58:3.

Ling, G. N., and C. L. Walton. 1976. What retains water in living cells? Science 191:293.

Metzler, D. E. 1977. The chemical reaction of living cells. Academic Press. First Edition.

Miller, E. J. 1973. A review of biochemical studies on the genetically distinct collagens of the skeletal system. Clin. Orthop. Relat. Research 92:260.

Mise, J. I. 1972. Factors affecting meat purchases and consumer acceptance of ground beef. Southern Cooperative Series Bull. 173:1.

Moledina, K. H., J. M. Regenstein, R. C. Baker, and K. H. Steinkrauss. 1977. Effects of antioxidants and chelators on the stability of stored mechanical deboned flounder meat from racks after filleting. J. Food Science 42:759.

Pabst, H. J. 1979. The hamburger phenomenon. Reciprocal Meat Conference Proceedings 32:17.

Piez, K. A. 1967. Soluble collagen and the components resulting from its denaturation. In "Treatise on Collagen" (G. N. Ramachandran, ed.), Vol. 1, p. 210. Academic Press, New York.

Ramachandran, G. N., and C. Ramakrishnan. 1976. Molecular structure. In: "Biochemistry of Collagen" (G. N. Ramachandran and R. Reddi, eds.), p. 45. Plenum, New York.

Reissmann, T. L., and J. Nichols. 1960. Collagen article and manufacture thereof. U.S. Patent 2,919,999.

Robinson, R. F. 1972. What is the future of textured protein products? Food Technology 26(5):59.

Root, L. 1978. Drover's J. Roll Call 32:64.

Sakakibra, S., K. Inouye, K. Shudo, Y. Kishida, Y. Kobayashi, D. J. Prockop. 1973. Synthesis of (Pro-Pro-Gly)n of defined molecular weights. Evidence for the stabilizing of collagen triple helix by hydroxyproline. Biochemistry Biophysical. Acta 303:198.

Salwin, H., Bloch, I. K., and Mitchel, J. H., Jr. 1955. Rapid determination of fat in meat products. J. Agricultural Food Chemistry 3:588.

Schalk, D. H. 1981. The use of bovine hide in coarse bologna. M.S. thesis, Oklahoma State University, Stillwater, Oklahoma.

Schubert, M., and D. Hamerman. 1968. "A primer on connective tissue biology," p. 5. Lea & Febiger, Philadelphia, Pennsylvania.

Sebranek, J. G., P. N. Sang, D. G. Topel, R. E. Rust, and A. A. Kraft. 1978. Influence of liquid nitrogen, liquid carbon dioxide and mechanical freezing on sensory properties of ground beef patties. J. Food Science 43:842.

Sebranek, J. G., P. N. Sang, D. G. Topel, and R. E. Rust. 1979. Effects of freezing methods and frozen storage on chemical characteristics of ground beef patties. J. Animal Science 48:1101.

Slutskii, L. I., and B. Z. Simkhovich. 1980. Molecular heterogeneity of collagen. Usp. Sovrem. Biology 89(1):141; Chemical Abstract 92:192772x.

Snyder, H. E. 1964. Measurement of discoloration in fresh beef. J. Food Science 29:535.

Steel, R. G., J. H. Torrie. 1980. Principles and procedures of statistics: A biometrical approach. McGraw Hill. Second edition.

Talty, R. D. 1969. Method of preparing edible tubular collagen casings. U.S. Patent 3,425,847.

Tanzer, M. L. 1973. Cross-linking of collagen. Science 180:561.

Tanzer, M. L. 1976. Cross-linking. In "Biochemistry of Collagen" (G. N. Ramachandran and A. H. Reddi, eds.), p. 137, Plenum, New York.

Tappel, A. L. 1952. Linoleate oxidation catalyzed by hog muscle and adipose tissue extracts. Food Research 17:550.

Tappel, A. L. 1953. Oxidative rancidity in food products. I. Linoleate oxidative catalized by hemin, hemoglobin and cytochrome C. Food Research 18:560.

Tappel, A. L. 1955. Unsaturated lipid oxidation catalyzed by hematin compounds. J. Biological Chemistry 217:721.

Turgot, V. A., M. Komanowsky, and H. I. Sinnamon. 1978. Comminuted collagen. Estimated cost of commercial production. Food Technology 32(4):48.

USDA. 1972. Using new foods to improve USDA child nutrition programs. Food and Nutrition Service, Nutrition and Technical Services staff. Mimeographed Report, April, 1972. Washington, D.C.

Weir, C. 1960. Palatability characteristics of meat. The Science of Meat and Meat Products. W. H. Freeman and Company.

Wells, L. H., B. W. Berry, and L. W. Douglas. 1980. Effects of grinding and mechanical desinewing in the manufacture of beef patties using conventional chilled and hot boned and rapidly chilled mature beef. J. Food Science 45:163.

Whilding, M. D. 1974. Textured proteins in meat and meat-like products. J. American Oil Chemists Society 51:128.

Whitmore, R., A. Booth, J. Naghski, and C. Swift. 1975. Digestibility and safety of limed hide collagen in rat feeding experiments. J. Food Science 40:101.

Woodhead-Galloway, J. Hukins, and J. S. Wray. 1975. Closest packing of two-stranded coiled coils as a model for the collagen fibril. Biochemistry Biophysical Research Commun. 64:1237.

APPENDIX

TABLE VIII

ANALYSIS OF VARIATION FOR PERCENT FAT IN GROUND BEEF PATTIES

Source	DF	Sum of Squares	Mean Squares	F Value	PR>F
Total	44	5.65			
Block	4	0.80	0.20	1.97	0.19
Collagen Level	2	0.67	0.33	3.31	0.09
Experimental Error	8	0.82	0.10		
Sampling Error	30	3.52			

TABLE IX

ANALYSIS OF VARIATION FOR PERCENT PROTEIN IN GROUND BEEF PATTIES

Source	DF	Sum of Squares	Mean Squares	F Value	PR>F
Total	44	5.65			
Block	4	0.80	0.20	1.97	0.19
Collagen Level	2	0.67	0.34	3.31	0.09
Experimental Error	8	0.82	0.10		
Sampling Error	30	3.35	0.11		

TABLE X

ANALYSIS OF VARIATION FOR PERCENT MOISTURE IN GROUND BEEF PATTIES

Source	DF	Sum of Squares	Mean Squares	F Value	PR>F
Total	44	19.84			
Block	4	5.43	1.36	2.51	0.13
Collagen Level	2	0.92	0.46	0.83	0.47
Experimental Error	8	4.31	0.54		
Sampling Error	29	9.19			

TABLE XI

ANALYSIS OF VARIATION FOR MOISTURE IN COLLAGEN

Source	DF	Sum of Squares	Mean Squares	F Value	PR>F
Total	14	821.70			
Can No.	4	235.90	58.98	.01	0.45
Error	10	585.80	58.58		

TABLE XII

ANALYSIS OF VARIATION FOR
PROTEIN IN COLLAGEN

Source	DF	Sum of Squares	Mean Squares	F Value	PR>F
Total	14	6.73			
Can No.	4	1.81	0.45	0.92	0.49
Error	10	4.91	0.49		

TABLE XIII

ANALYSIS OF VARIATION FOR
FAT IN COLLAGEN

Source	DF	Sum of Squares	Mean Squares	F Value	PR>F
Total	14	0.012			
Can No.	4	0.014	0.003	10.85	0.001*
Error	10	0.003	0.003		

*Significant ($P<0.05$)

TABLE XIV

ANALYSIS OF VARIATION FOR FLAVOR

Source	DF	Sum of Squares	Mean Squares	F Value	PR>F
Total	275	341.08			
Whole Units	42	18.11			
Blocks	4	2.18	0.55	1.34	0.32
Panelists	8	3.62	0.45	1.09	0.80
Error (a)	30	12.31	0.41		
Subunits	233	322.97			
Collagen Level	2	232.62	116.31	294.96	0.0001*
Storage Week	2	0.77	0.39	0.97	0.38
Collagen x Storage	4	0.86	0.22	0.54	0.70
Error (b)	225	88.72	0.39		

*Significant ($P<0.05$)

TABLE XV

ANALYSIS OF VARIATION FOR JUICINESS

Source	DF	Sum of Squares	Mean Squares	F Value	PR>F
Total	275	232.16			
Whole Units	42	43.58			
Blocks	4	6.03	1.51	2.21	0.09
Panelists	8	19.57	2.45	3.54	0.005*
Error (a)	30	17.98	0.60		
Subunits	233	188.59			
Collagen Level	2	12.68	6.34	8.83	0.0002*
Storage Week	2	2.17	1.09	1.51	0.22
Collagen x Storage	4	12.15	3.04	4.23	0.0025*
Error (b)	225	161.59	0.72		

*Significant ($P<0.05$)

TABLE XVI

ANALYSIS OF VARIATION FOR TEXTURE

Source	DF	Sum of Squares	Mean Squares	F Value	PR>F
Total	275	353.49			
Whole Units	42	24.66			
Blocks	4	4.08	1.02	2.06	0.11
Panelists	8	3.90	0.49	0.87	0.55
Error (a)	30	16.68	0.56		
Subunits	233	328.83			
Collagen Level	2	207.08	103.54	203.19	0.0001*
Storage Week	2	0.14	0.07	0.13	0.87
Collagen x Storage	4	6.96	1.74	3.41	0.01
Error (b)	225	114.65	0.51		

*Significant ($P<0.05$)

TABLE XVII

ANALYSIS OF VARIATION FOR OVERALL ACCEPTABILITY

Source	DF	Sum of Squares	Mean Squares	F Value	PR>F
Total	275	371.69			
Whole Units	42	24.83			
Blocks	4	0.18	0.05	0.16	0.96
Panelists	8	4.58	0.57	0.83	0.58
Error (a)	30	20.07	0.67		
Subunits	233	346.88			
Collagen Level	2	239.52	119.76	203.19	0.0001*
Storage Week	2	0.13	0.07	0.13	0.88
Collagen x Storage	4	1.66	0.42	3.41	0.01
Error (b)	225	105.57	0.47		

*Significant ($P<0.05$)

TABLE XVIII

ANALYSIS OF VARIATION FOR COOK LOSS

Source	DF	Sum of Squares	Mean Squares	F Value	PR>F
Total	134	65.22			
Blocks	4	6.10	1.52	4.17	0.004*
Treatments	8	2.91	0.36		
A (Collagen Level)	2	1.11	0.56	1.52	0.22
B (Storage Weeks)	2	0.27	0.14	0.37	0.69
A x B	4	1.53	0.38	1.05	0.39
Block x Treatments	32	23.34			
Block x A	8	4.74	0.59	1.62	0.13
Block x B	8	1.81	0.23	0.62	0.76
Block x A x B	16	16.79	1.05	2.87	0.00
Experimental Error	90	32.87	0.37		

*Significant ($P<0.05$)

TABLE XIX

ANALYSIS OF VARIATION FOR HUNTER L VALUE OF UNCOOKED PATTY

Source	DF	Sum of Squares	Mean Squares	F Value	PR>F
Total	137	1166.16			
Block	4	66.70	16.67	4.65	0.005
Treatment	8	634.82	79.35		
A (Collagen Level)	2	510.67	263.67	71.21	0.0001*
B (Storage Week)	2	73.58	36.79	10.26	0.0004*
A x B	4	50.57	12.64	3.53	0.0171
Experimental Error	32	114.74	3.59		
Sampling Error	93	349.90	3.76		

*Significant ($P<0.05$)

TABLE XX

ANALYSIS OF VARIATION FOR HUNTER a
VALUE OF UNCOOKED PATTY

Source	DF	Sum of Squares	Mean Squares	F Value	PR>F
Total	137	8111.91	59.21		
Block	4	228.13	57.03	0.90	0.48
Treatment	8	446.15	55.77		
A (Collagen Level)	2	137.90	68.95	1.09	0.35
B (Storage Week)	2	124.27	62.13	0.98	0.39
A x B	4	183.98	45.99	0.72	0.58
Experimental Error	32	2032.13	63.50		
Sampling Error	93	5405.50	58.12		

TABLE XXI

ANALYSIS OF VARIATION FOR HUNTER b
VALUE OF UNCOOKED PATTY

Source	DF	Sum of Squares	Mean Squares	F Value	PR>F
Total	137	9738.16			
Block	4	279.22	69.80	0.95	0.45
Treatment	8	456.26	57.03		
A (Collagen Level)	2	121.82	60.91	0.83	0.44
B (Storage Week)	2	83.54	41.77	0.53	0.57
A x B	4	250.90	62.72	0.86	0.50
Experimental Error	32	2344.25	73.26		
Sampling Error	93	6658.43	71.60		

TABLE XXII

ANALYSIS OF VARIATION FOR HUNTER L VALUE OF COOKED PATTY

Source	DF	Sum of Squares	Mean Squares	F Value	PR>F
Total	137	2344.26			
Block	4	32.83	8.21	0.42	0.79
Treatment	8	201.58	25.20		
A (Collagen Level)	2	42.74	21.37	1.09	0.35
B (Storage Week)	2	98.63	49.31	2.51	0.10
A x B	4	60.21	15.05	0.77	0.55
Experimental Error	32	627.98	19.62		
Sampling Error	93	1481.86	15.93		

TABLE XXIII

ANALYSIS OF VARIATION FOR HUNTER a
VALUE OF COOKED PATTY

Source	DF	Sum of Squares	Mean Squares	F Value	PR>F
Total	137	66.14			
Block	4	4.72	1.18	2.03	0.11
Treatment	8	38.17	4.77		
A (Collagen Level)	2	10.19	5.09	8.78	0.0009*
B (Storage Week)	2	24.56	12.28	21.16	0.0001*
A x B	4	3.42	0.85	1.47	0.23
Experimental Error	32	18.57	0.58		
Sampling Error	93	4.67	0.050		

*Significant (P<0.05)

TABLE XXIV

ANALYSIS OF VARIATION FOR HUNTER b
VALUE OF COOKED PATTY

Source	DF	Sum of Squares	Mean Squares	F Value	PR>F
Total	137	24.13			
Block	4	3.09	0.78	2.59	0.06
Treatment	8	3.00	0.38		
A (Collagen Level)	2	0.32	0.16	0.54	0.59
B (Storage Week)	2	0.47	0.24	0.80	0.46
A x B	4	2.21	0.55	1.85	0.14
Experimental Error	32	9.53	0.30		
Sampling Error	93	8.50	0.09		

TABLE XXV

ANALYSIS OF VARIATION FOR SHEAR FORCE

Source	DF	Sum of Squares	Mean Squares	F Value	PR>F
Total	134	82.84			
Blocks	4	17.38	4.35	8.50	0.0001*
Treatments	8	28.84			
A (Collagen Level)	2	3.87	1.90	3.26	0.04
B (Storage Week)	2	20.60	10.30	18.89	0.0001*
A x B	4	4.36	1.12	2.00	0.12
Experimental Error	32	18.53	0.58		
Sampling Error	90	18.02	0.19		

*Significant ($P<0.05$)

TABLE XXVI

ANALYSIS OF VARIATION FOR TBA VALUE OF UNCOOKED BEEF PATTIES

Source	DF	Sum of Squares	Mean Squares	F Value	PR>F
Total	134	297.44			
Block	4	257.44	64.36	94.03	0.0001*
Treatment	9	16.89	1.88		
A (Collagen Level)	2	16.25	8.13	11.88	0.0001*
B (Storage Weeks)	2	0.20	0.10	0.14	0.87
A x B	4	0.44	0.11	0.16	0.96
Experimental Error	32	21.90	0.68		
Sampling Error	90	1.21			

*Significant ($P<0.05$)

TABLE XXVII

ANALYSIS OF VARIATION FOR TBA VALUE OF COOKED BEEF PATTIES

Source	DF	Sum of Squares	Mean Squares	F Value	PR>F
Total	134	374.55			
Block	4	317.09	79.27	87.22	0.0001*
Treatment	9	26.80	2.98		
A (Collagen Level)	2	15.73	7.87	8.65	0.0010*
B (Storage Weeks)	2	10.33	5.17	5.68	0.008
A x B	4	0.74	0.18	0.20	0.93
Experimental Error	32	29.08	0.91		
Sampling Error	90	1.57			

*Significant ($P<0.05$)

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